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1: J Cell Biol 1996 Sep;134(6):1543-9

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## The cell density factor CMF regulates the chemoattractant receptor cAR1 in Dictyostelium.

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Starving Dictyostelium cells aggregate by chemotaxis to cAMP when a secreted protein called conditioned medium factor (CMF) reaches a threshold concentration. Cells expressing CMF antisense mRNA fail to aggregate and do not transduce signals from the cAMP receptor. Signal transduction and aggregation are restored by adding recombinant CMF. We show here that two other cAMP-induced events, the formation of a slow dissociating form of the cAMP receptor and the loss of ligand binding, which is the first step of ligand-induced receptor sequestration, also require CMF. Vegetative cells have very few CMF and cAMP receptors, while starved cells possess approximately 40,000 receptors for CMF and cAMP. Transformants overexpressing the cAMP receptor gene cAR1 show a 10-fold increase of [3H]cAMP binding and a similar increase of [125I]CMF binding; disruption of the cAR1 gene abolishes both cAMP and CMF binding. In wild-type cells, downregulation of cAR1 with high levels of cAMP also downregulates CMF binding, and CMF similarly downregulates cAMP and CMF binding. This suggests that the cAMP binding and CMF binding are closely linked. Binding of approximately 200 molecules of CMF to starved cells affects the affinity of the majority of the cAR1 cAMP receptors within 2 min, indicating that an amplifying mechanism allows one activated CMF receptor to regulate many cARs. In cells lacking the G-protein beta subunit, cAMP induces a loss of cAMP binding, but not CMF binding, while CMF induces a reduction of CMF binding without affecting cAMP binding, suggesting that the linkage of the cell density-sensing CMF receptor and the chemoattractant cAMP receptor is through a G-protein.

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